Day 30 - Monday- 07/14/14

As a team, we hosted 30 10-12 year old girls from the Women in Science Day camp. The girls rotated through three stations. In the conference room, the girls put together and paper DNA puzzle and tried to decode their codons with a codon chart. In the lab, the girls learned about bacteria that can detect pollution in water and did a sniff test for pollutants. Water that was contaminated with antibiotics smelled like wintergreen while water with heavy metal contamination smelled like bananas. They also made their own non-toxic rainbow gels with food coloring to learn about pipetting and gel electrophoresis. A curriculum for the event can be found in the women in science folder on the googledrive.

At the end of the day after the girls left, Corbyn and Shawna liquid cultured 5 colonies from the CAEV biobrick plate. We chose the three colonies that had shown a band during the colony PCR that appeared the right size (colonies 1,4,and 9) and we also used colony 2 which seemed unsuccessful for the colony PCR and picked a random one from the plate that was not colony PCR'd.

Day 31 - Tuesday- 07/15/14

- Alex and Chloe did another run on the plate reader with the new interlab ligations, using colonies E and B from plate 2.3 (low promoter) and colonies H and G from plate 1.6 (high promoter). On the first run, the readings for H did not match the readings for H even though the two cultures were supposed to be identical based on the gel ran last Friday. However, upon performing a second run with newly filled wells, the readings for H and G matched, pinning the inconsistency as a pipetting error. The readings for colony G and H were abnormally high, almost quadruple the readings for 17K (which was supposed to have the same value), so all 4 samples used on this plate were sent for sequencing. The results can be found in the interlab study folder.
- Mike prepared a new 7C miniprep and large 7C and 23C digests for gel purification. The digests were prepared according to the table below. Both tubes were put in the thermocycler, which was programmed to run at 37^{o}C for 1 hour followed by 80^{o}C for 20 minutes. Following the restriction digest, the DNA was run on a gel and then gel purified according to the gel purification protocol.

  Gel Purification Digest Set Up

	DNA Concentration (ng/uL)	5ug DNA volume (uL)	EcoRI Volume (uL)	XbaI Volume (uL)	SpeI Volume (uL)	BSA Volume (uL)	NEB Buffer 2 Volume (uL)	dH2O Volume (uL)	CIP Volume (uL)
7C	153.8	32.5	2	0	2	2	5	6.5	0

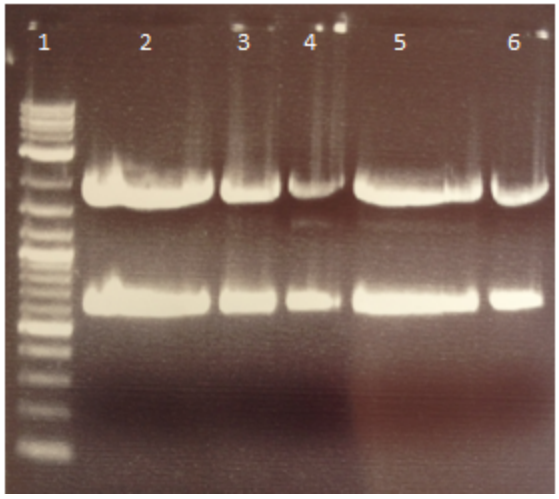
23C	365.8	13.67	2	2`````````` ``	0	2	5	23.33	2

While Mike worked on the minipreps, digests, and gel purification, Kayla tried the agglutination quantification again according to the protocol designed last Friday. The full protocol is title “Original Agglutination Quantification Protocol” and can be seen in the protocol folder. The initial OD of the control, the 1:200 antibody dilution, the 1:2400 antibody dilution, and the 1:200 non-specific antibody dilution were measured. The samples were then rotated for an hour and then divided in half among 30 micron spin columns to be spun down at 1500 rpm for 1 minute. The final ODs were then measured and recorded. The results can be seen in the table below. According to the final ODs, either no agglutination took place or the clumps were able to pass through the columns. To test whether or not the agglutination actually took place, more columns will be ordered and the samples will be rotated overnight to allow more time for the agglutination to occur. Some more tests may have to be done to optimize this agglutination quantification protocol.

Agglutination Quantification Results

	Liquid Culture Volume (uL)	5% BSA in PBS Volume (uL)	Antibody	Antibody Volume (uL)	Antibody Dilution	OD_o	OD_f	$\%OD_o$	$\%OD_o$: $\%OD_o$(A)
A	600	600	none	0	0	0.773	0.647	83.7%	0
B	600	600	specific	6	1:200	0.783	0.627	80.1%	-3.6%
C	600	600	specific	0.5	1:2400	0.772	0.667	86.4%	2.7%
D	600	600	non-specific	6	1:200	0.759	0.672	88.5%	4.8%

- Corbyn and Shawna miniprepped all 5 potential CAEV biobricks that they had liquid cultured the night before. Each miniprep was nanodropped and the concentrations were acceptable (they can be seen in the miniprep document in the igem folder). We then sent of all 5 of the potential CAEV biobricks as well as the promoter (5A) for sequencing. We sent the promoter because of the incident where Corbyn and I thought we created an RBS-Promoter construct but a double terminator was actually ligated to the RBS. We then prepared a 50uL (E-P) test digest of all 5 potential CAEV biobricks to test if they were in fact correct. We ran the digests on a 1% gel at 70V for 1 hour, and the result can be seen below. As you can see, some of the lanes are the double wide wells, this is only because we used a previously prepared gel that was intended for gel purification. The bands at roughly 600 for all 5 samples indicate there is a strong possibility that the CAEV biobrick was successfully created.



Lane Number	Contents
1	ladder
2	CAEV BB 1
3	CAEV BB 2
4	CAEV BB 3
5	CAEV BB 4
6	CAEV BB 5

After the gel, we were fairly confident that the CAEV biobrick had been created, so we set up a 50 uL digest of CAEV BB 1 with X-P and the sequence confirmed BCLA biobrick 5 with S-P with the intention of ligating them together the following day. We placed the digests in the 37 degree water bath overnight.

Day 32 - Wednesday- 07/16/14

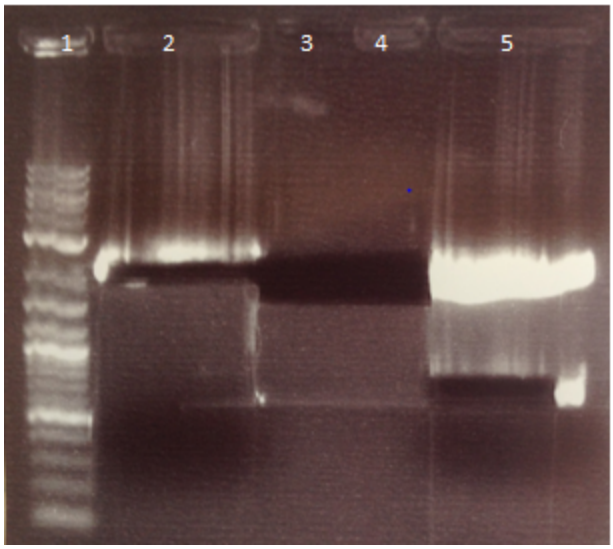
- Alex and Chloe examined the sequencing data and confirmed that 1.6 H, 1.6 G, 2.3 B, 2.3 E, and 17K were exactly what they should be. Because the sequences are correct, all 6 1.6 liquid cultures were back diluted and grown to an OD of 0.9 then run on the plate reader to check if there was any difference between the back-diluted cultures and overnight liquid cultures. A transformation of the Promoter+RBS+BCLA+CAEV+DT construct was also prepared and left to incubate overnight.

- Kayla started the day by running the 7C and 23C digests on a gel in two large wells. The gel was run at 72V for an hour, and then placed on the UV light table so the DNA bands could be cut out. Once the bands were cut, they were placed in two separate conical tubes and the gel purification protocol was followed. The rundown of the gel purification method is in the Week 7 directory, under "**Bananarse Gel Purification.**" The DNA concentrations of the purification products were measured. The concentration of the 7C extract was 27 ng/uL and the concentration of the 23 C extract was 21 ng/uL. Following the gel purification, the products were ligated together in three different reactions with one control. A summary table of the four ligation tubes can be seen below. To end the day, four transformations were performed with the ligation products.

7/16/14 Ligations

	7C DNA (uL)	23C DNA (uL)	dH2O (uL)	Ligase Buffer (uL)	Ligase (uL)
1	2	2	13	2	1
2	1	1	15	2	1
3	1	2	14	2	1
4 (Control)	0	2	15	2	1

- Corbyn and Shawna started the day by CIPping the BCLA that had been digested with S-P overnight. Then, we ran the CIPped S-P digest of BCLA along with the X-P digest of CAEV on a 1% gel in order to gel purify it. We loaded the entire digest (50uL) into the larger double wide wells and ran at 70V for an hour . The correct fragments were then cut out using the light table, and gel purified using the kit. Below, a picture of the gel after the inserts were cut out can be seen.



Lane #	Contents
1	ladder
2	50uL BCLA S-P (CIP)
3	Empty
4	Empty
5	50uL CAEV X-P

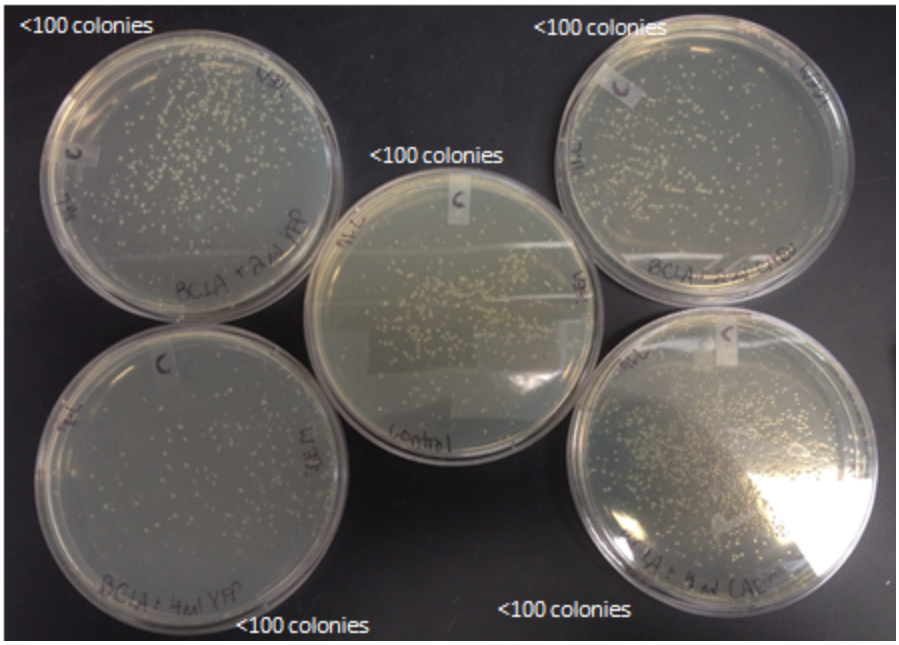
We then ligated the gel purified S-P BCLA to YFP (X-P) as well as CAEV (X-P). We used two different ratios of insert to BCLA, and the contents of each along with their labeled code can be seen below.

Code	Gel purified S-P BCLA (uL)	Insert	dH2O (uL)
B+Y2	2	2uL YFP	11
B+Y4	2	4uL YFP	9
B+C2	2	2uL CAEV	11
B+C4	2	4uL CAEV	9

When the ligations were complete, we transformed all 4 ligations as well as just the CIPped BCLA S-P gel purified vector as a control. The transformations were all plated on chloramphenicol plates and placed in the warm room overnight.

Day 33 - Thursday - 07/17/14

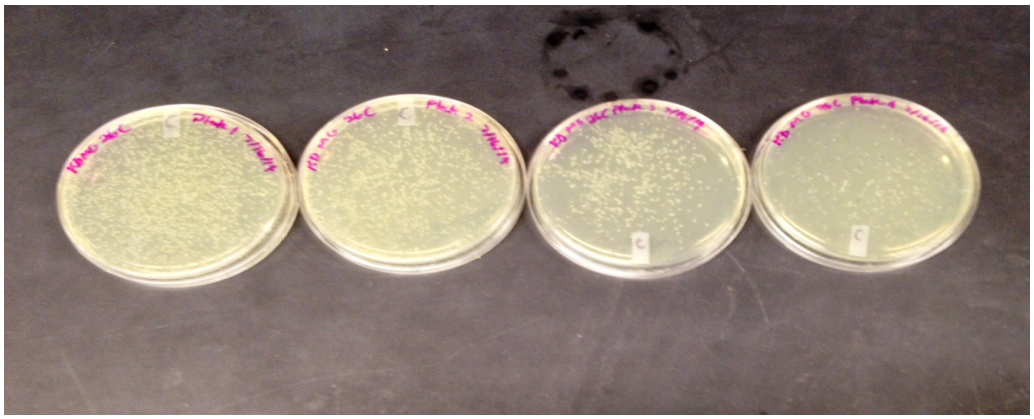
- Shawna and Corbyn started the day by collecting the BCLA+CAEV and BCLA+YFP ligation plates from the previous day. Each of the five plates had over 100 colonies (see picture below).



At the end of the day, 6 colonies were selected from both the B+C4 and the B+Y2 plate and liquid cultured overnight in the shaker. Next. a digestion of the promoter oligo was done with E

and S and the RBS oligo was digested with X and P. The digestion was run for 2 and a half hours and then heat killed for 20 minutes. A table top ligation was performed using the two parts (RBS and Promoter) and pSB1A3 which was digested with E and P. Three ligations were done each with a different ratio of RBS and Promoter digests; 2RBS:2Promoter (tube 1), 1 RBS:2 Promoter (tube 2), and 2RBS:1Promoter (tube 3). These three ligations were then transformed into competent cells and plated on ampicillin plates which were then placed in the warm room overnight.

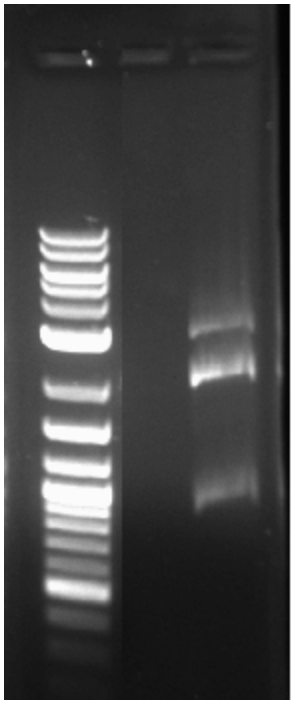
- Kayla and Mike checked the 26C transformation plates. All four plates, including the control, had over 100 colonies on them. A picture of the plates can be seen below. 7/17/14 Transformation Results



Because the control had more than 100 colonies, 8 colonies were selected from the plate with the most growth for liquid culture. In addition to liquid culturing for minipreps, the annealed promoter, J23100, was digested with EcoRI and SpeI and ligated into pSB1C3 digested with EcoRI and PstI. A transformation was then performed with the ligation product.

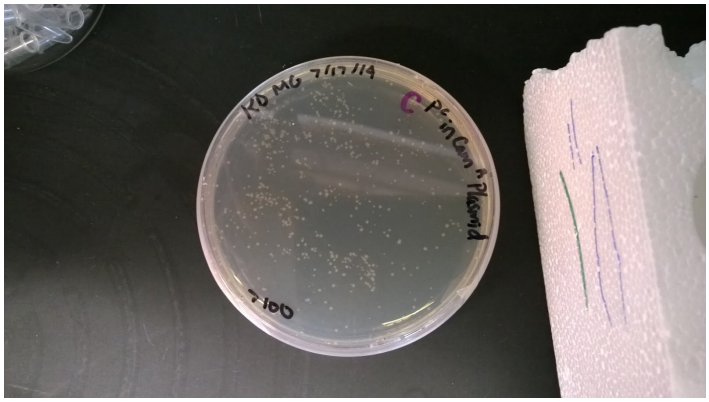
Day 34 - Friday- 07/18/14

- Alex and Chloe back diluted the overnight 25K culture to 1:20 as well as miniprepped the remaining culture. The purified plasmid and chlor linearized vector were cut with E and P and a gel was run on the digested plasmid. The insert was CIP treated.



Lane	Contents
1	Ladder
2	25K (Final Construct)

- Kayla and Mike retrieved the transformation plate containing the constitutive promoter, it contained more than 100 colonies. A picture is seen below:



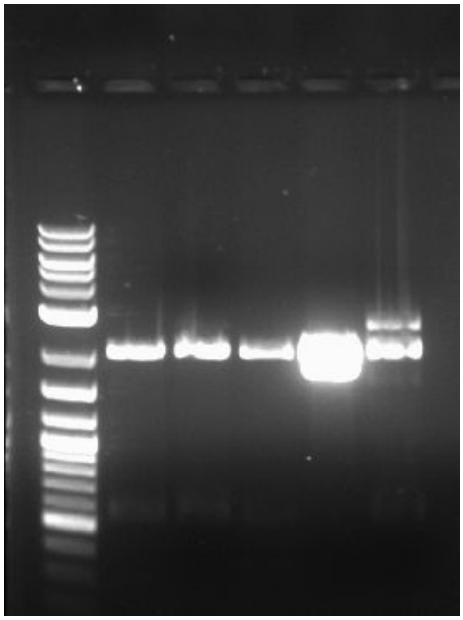
The plate was then placed in the cold room.
Minipreps were then made for all 8 liquid cultures of the 26C colonies. All of the transformations showed good curves, their respective concentrations are below:

Tube	DNA Concentration (ng/uL)
26C MP1	221.6
26C MP2	190.9

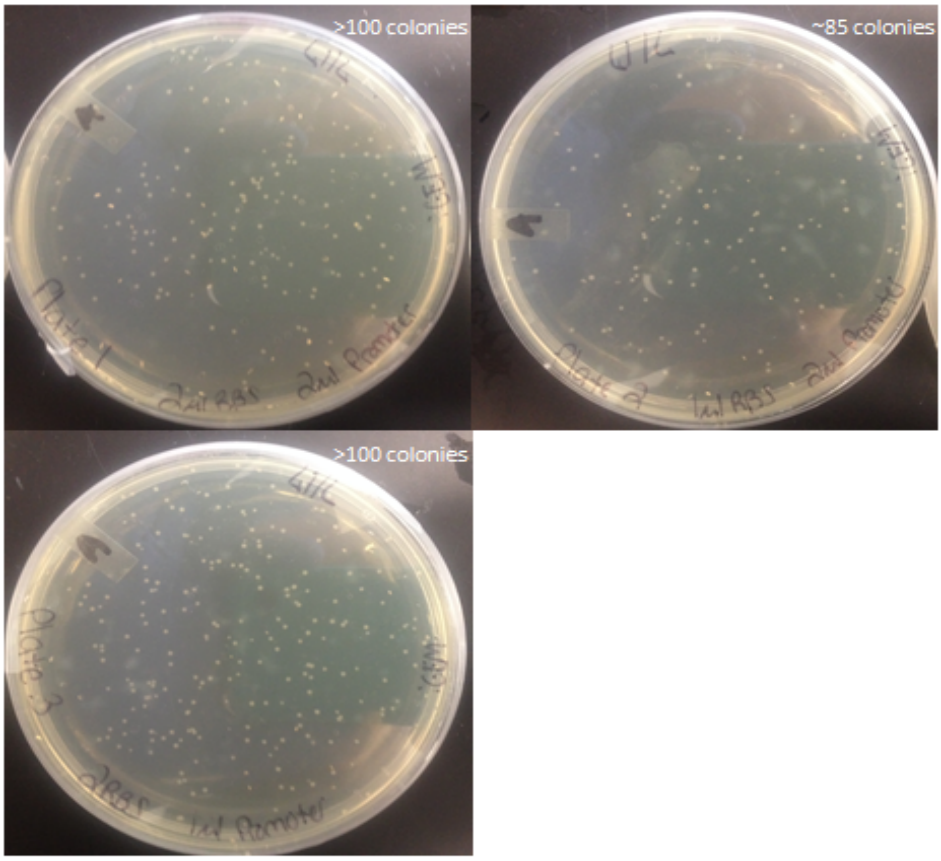
26C MP3	159.2
26C MP4	571.8
26C MP5	205.2
26C MP6	308.0
26C MP7	260.0
26C MP8	240.7

The first five minipreps were test digested and run on a gel at 80V for 45 minutes. All eight minipreps could not be test digested because all of the PstI was used on the first five tubes. The picture of the gel can be seen below. The 2-log ladder is in lane 1, and minipreps 1-5 are in order in lanes 2-6. Because minipreps 4 and 5 each had 2 bands around 2 kb, they were both sent off for sequencing.

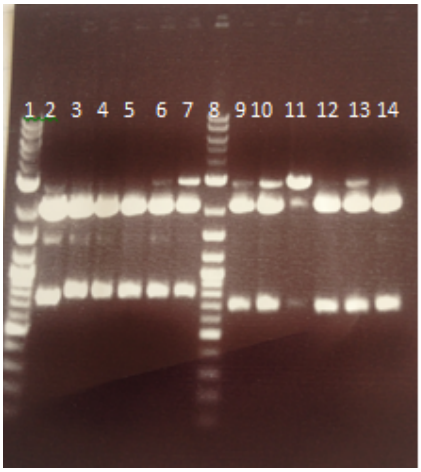
7/17/14 Gel



- Corbyn and Shawna started the day by collecting the Promoter+RBS plates. Plate 1 and 3 had more than 100 colonies, however plate 2 had roughly 85 colonies (see pictures below)



We then miniprepped the 6 BCLA+CAEV and 6 BCLA+ YFP liquid cultures and subsequently nanodropped the samples to check the DNA concentration. In order to test if our ligations were successful, we digested all 12 samples with S+X (due to the stock of E and P being gone) and ran them on a 1% gel for 1 hour at 80V. A picture of the resulting gel with labeled lanes can be seen below, and as you can see it appeared as if most of the samples had a band at the correct size (BCLA+CAEV between 700- 800 and
BCLA+YFP around 900).



Lane	Contents
1	Ladder
2	BCLA+YFP 1
3	BCLA+YFP 2
4	BCLA+YFP 3
5	BCLA+YFP 4
6	BCLA+YFP 5
7	BCLA+YFP 6
8	Ladder
9	BCLA+CAEV 1
10	BCLA+CAEV 2
11	BCLA+CAEV 3
12	BCLA+CAEV 4
13	BCLA+CAEV 5
14	BCLA+CAEV 6

We then sent BCLA+YFP sample 4 and 5 as well as BCLA+CAEV sample 4 and 5 for sequencing, only using VF. Over the weekend, we checked the sequencing and found that the

ligations were in fact successful and the constructs were correct. We then updated the tube and miniprep library, BCLA+CAEV in PSB1C3 is 13C4 and 13C5 (from a previous attempt) and BCLA+ YFP is 27C4 and 27C5.